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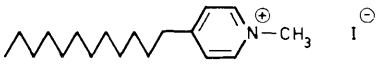
SUMMARY

This thesis describes a multidisciplinary study of the aggregation behavior of 1-methyl-4-dodecylpyridinium iodide (1), 17-(4-N-methylpyridinium)tritriacontane iodide (2), and 3,5-dicarbo-n-hexadecyloxy-1-methylpyridinium iodide (3) and of the properties of the corresponding aggregates in apolar organic solvents and in water. The characterization of the liquid-crystalline phase of these amphiphiles in the neat state has also been described. These



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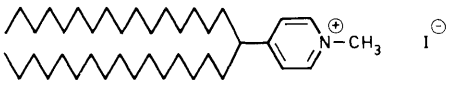

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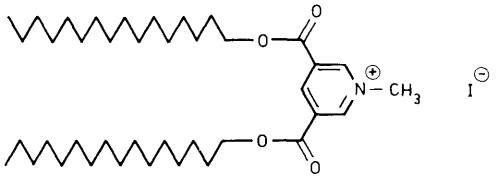

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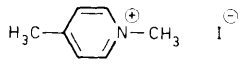
amphiphiles were chosen for investigation because they carry a 1-methylpyridinium iodide head group. This functionality exhibits a long-wavelength intramolecular charge-transfer (CT) absorption. The position of the CT band is very sensitive to changes in the polarity of the medium. Linear correlations were observed upon plotting the transition energy of the CT band of monomeric 1 and of monomeric model compounds for 2 and 3 in media of different polarity against Kosower's Z values. In this way the position of the CT band of 1-3 in different states of aggregation can be expressed in the micropolarity parameter Z.

The 1-methylpyridinium iodide head group was shown to be a valuable *intrinsic* microscopic polarity reporter, upon aggregation of the amphiphiles 1-3 in water and in apolar organic solvents. This type of approach has an advan-

tage over that employing *extrinsic* reporters, since the position of the reporter is exactly known, and the aggregated structure is not disturbed by the presence of the reporter.

In Chapter 1 a short and general introduction is given on the molecular structure of amphiphiles and the possible types of organization of amphiphilic molecules both in solution and in some mesophases.

Chapter 2 describes a study of the aggregation of 1-3 and of 1,4-dimethylpyridinium iodide (4) in dichloromethane (CH_2Cl_2) and chloroform ($CHCl_3$). Amphiphiles 2 and 3 were also studied in tetrachloromethane (CCl_4), benzene (C_6H_6), and cyclohexane (c-C_6H_{12}).



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Vapor pressure osmometric measurements showed an interesting diversity in aggregation behavior. Depending on amphiphilic structure, solvent, concentration, and temperature, the size of the aggregates varied from triple ions to reversed micelles with apparent number averaged aggregation numbers up to $\bar{N}_{NA}$ = 14-15. The tendency for aggregation increases in the order of 3 < 2 < 1 < 4, indicating that increasing alkyl-chain length and introduction of a second alkyl chain in the amphiphile exert negative effects on the aggregation process. Association is more pronounced in solvents of lower polarity (CH_2Cl_2 < $CHCl_3$ < C_6H_6 < CCl_4 < c-C_6H_{12}), at higher concentrations, and at lower temperatures.

Optical absorption measurements on the CT band revealed a gradual increase of the transition energy with increasing stoichiometric concentration of the compound. This indicates that dipole-dipole interaction between the head groups is one of the factors involved in the aggregation process. The observed shifts were interpreted in terms of increasing micropolarity near the heads. Values varying between Z = 65.9 for triple ions formed from 2 in CH_2Cl_2, and Z = 70.7 (corresponding to a solvation sphere in acetonitrile) for trimers formed from 3 in $CHCl_3$ were found. In the reversed micelle ($\bar{N}_{NA}$ = 14-15) formed from 2 in c-C_6H_{12}, the observed Z value (68.8) is very similar to that defined for N,N-dimethylformamide as the solvent (Z = 68.5). Addition of water to these reversed micellar solutions is accompanied by a pronounced increase of the transition energy, indicating that water is solubilized near the head groups. For reversed micellar solutions of 2 in C_6H_6 saturated with water this leads to Z = 74.5, which corresponds to the Z value of the solvation sphere of 2-propanol.

Preliminary near-infrared measurements suggest that the water molecules in the water pools of the reversed micelles from 2 in c-C_6H_{12} are mutually stronger hydrogen bonded than in bulk water.

Two new Z values were derived: Z = 61.5 ± 0.2 for CCl_4 and Z = 62.5 ± 0.2 for C_6H_6.

In the solvents CH_2Cl_2 and $CHCl_3$ conductivity measurements provided dissociation constants for ion pairs and triple ions formed from 1-4 (K_D and K_3, respectively). The K_D values increase in the order of 4 < 3 < 2 < 1, whereas the K_3 values followed a different sequence (2 < 4 < 1 < 3). The apparent equivalent conductances at infinite dilution clearly indicate the reduced mobility of the pyridinium cation upon introduction of more and longer alkyl chains.

A study of the aggregation of amphiphile 1 in water to form micelles has been described in Chapter 3. The critical micellar concentrations (CMC's) were determined conductometrically in the presence of different concentrations of several sodium salts. The observed decrease of the CMC at increasing salt concentration followed the total counterion concentration at the CMC (*i.e.* the Shinoda equation). The salt effect was more pronounced in the order of: Cl^- < Br^- < NO_3^- < I^- < ^-OTs, which parallels the lyotropic series for anions.

CMC determinations by means of optical absorption measurements on the CT band of 1 in water were in accord with those determined from conductometry. The position of the CT band in micellar solutions of 1 is found at 286 ± 1 nm. This value indicates a reduced micropolarity in the Stern-layer as expressed in Z = 80.6 ± 0.2 (corresponding with a solvation sphere of 1 in ethanol). For monomeric 1 in water Z = 94.6. The reduction of micropolarity in the Stern-layer probably originates from the proximity of the hydrocarbon core and/or a dielectric saturation effect. Only a minor effect on the position of the CT band maximum was observed upon addition of several sodium salts (NaCl, NaBr, NaI, $NaNO_3$, and NaOTs). Values for the magnitude of the iodide counterion binding (B) were derived from the apparent molar extinction coefficients (B = 85 ± 2%). B increases gradually upon addition of iodide anions. The binding constant of added iodide ions in the Stern-layer was calculated to be K_I = 140 ± 20 l mol^{-1}. Addition of non-common anions resulted in a decrease of B, due to a competition of the anions for binding in the Stern-layer. This salt effect became more pronounced in the order of Cl^- < Br^- < NO_3^- < ^-OTs. The obtained results are discussed in terms of a reduction of charge density per surface area of the micelle, which leads to a lowering of Coulombic repulsions between the head groups.

Sonication of the double-chained amphiphiles 2 and 3 in aqueous solution leads to the formation of uni- and multilamellar vesciles, as revealed by electron microscopy. This study is described in Chapter 4. Both amphiphiles also form mixed vesicles with phosphatidyl choline. Leakage of entrapped carboxyfluorescein (CF) from the aqueous vesicle compartments was studied by fluorescence spectroscopy. CF molecules are rapidly released from vesicles formed from 2 at 0°C. Under the same conditions, CF molecules escape less rapidly from vesicles formed from 3. If the sonication of 3 is carried out at 50°C, leakage of CF is a slow process. These results are rationalized in terms of thermotropic phase transitions of the vesicle bilayer. The phase-transition temperatures have been determined using differential scanning calorimetry (DSC) measurements.

From optical absorption spectroscopic measurements on the CT band of the 1-methylpyridinium iodide head group, values for the micropolarity in the electrical double layer of the vesicles were determined. Vesicles formed from 2 showed Z = 64.2, which corresponds to a solvation sphere of dichloromethane, while vesicles formed from 3 show a somewhat less dramatic effect. Here a value of Z = 74.0 was obtained, corresponding with a solvation sphere slightly more apolar than that in 2-propanol. Interestingly, the reduction of the micropolarity, with respect to bulk water, is much more than that observed in the Stern-layer of micelles formed from 1. Close packing of the head groups within the vesicles and a concomitant reduced hydration of the head groups are invoked to explain these results.

Chapter 5 describes an application of the vesicles formed from the synthetic double-chained amphiphiles 2 and 3 to mimic a biological membrane. Now the corresponding chlorides (2' and 3', respectively) were used instead of the iodides. In this study the visual pigment bovine rhodopsin was incorporated into the vesicle bilayer. Under these conditions rhodopsin was found to exhibit the same absorption spectrum as in the intact biological membrane. It is, therefore, concluded that the chromophoric center of rhodopsin is not affected by reconstitution into these artificial membranes. The recovery of photosensitive rhodopsin in these vesicles was > 90%. Illumination of the reconstituted rhodopsin vesicles formed from 2' at 25°C showed metarhodopsin II as the first photointermediate, followed by the formation of metarhodopsin III, and finally all-*trans*-retinal. Metarhodopsin II is also the principal first detectable photointermediate after illumination of rhodopsin in vesicles from 2' at 16°C and in vesicles from 3' at 25°C. Metarhodopsin I was detected in the photolysis of reconstituted rhodopsin in vesicles formed from 2' at 1.5°C. Under

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these conditions the sequence of intermediates stopped at metarhodopsin II, as was shown by protonation of the Schiff's base bond. The maximal chemical regeneration capacity of fully bleached rhodopsin was found to be 70%. Incomplete regeneration probably finds its origin in aggregation of the apoprotein, leading to shielding of the active site.

Electron micrographs obtained by both negative-staining and freeze-fracturing techniques provide strong additional evidence for the incorporation of rhodopsin into the hydrocarbon part of the vesicle membrane. The heterogeneous distribution of rhodopsin over the vesicle membrane, as revealed from electron microscopy, was also indicated by the recovery and chemical regeneration determinations.

The results are discussed in relation to rhodopsin reconstitutions in phospholipid vesicles and rhodopsin in the rod outer segment membrane. The reconstitutions indicate that these vesicles successfully mimic essential environmental properties of the biological membrane.

Finally, in Chapter 6, a study of the thermotropic phase transitions in 1-3 and in several structurally related pyridinium amphiphiles is described. From a combination of differential scanning calorimetry, optical polarization microscopy, and X-ray diffraction, the thermotropic liquid-crystalline phases present in 1-3 and in the perchlorate derivative of 1 (indicated by compound number 14) were characterized. For amphiphile 1 and 14 a smectic-A phase was observed. The thermotropic liquid-crystalline state of 2 could be identified as a smectic-C phase. In this phase the amphiphiles are oriented tail to tail in the liquid bilayer. The tilt angle of the director was calculated to be *ca* 58^{o}. Using a least-squares refinement procedure on the X-ray diffraction pattern of the mesophase of amphiphile 3, the structure can be best reconciled with that of a smectic-H phase. The cell constants of the monoclinic lattice are: a = 8.6 Å, b = 4.8 Å, c = 51.8 Å, and $\beta = 132.5^{o}$. The results are discussed in relation to the calculated molecular dimensions obtained from space-filling molecular models. Probable arrangements of the organized amphiphiles in the particular smectic phase are proposed.